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RESEARCH ARTICLE

Optimizing the making of modified plantain flour by the mixed-culture of lactic acid bacteria and yeast

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ABSTRACT

Fermentation factors in the making of modified plantain flour must be optimized to obtain optimum conditions that produce the desired characteristics of flour/starch. In this research, we used mix-cultured lactic acid bacteria (LAB) and yeast for plantain fermentation. Response Surface Methodology (RSM) was used to explain the quantitative relationship between input and response variables. The design used is Combine D-optimal. The quantitative independent variables (X) selected were A = culture concentration (lower limit 2%; upper limit 6%) and B = fermentation time (lower limit 8 hours; upper limit 24 hours). Descriptive variables were C = type of plantain (C_1 = "tanduk" plantain; C_2 = "nangka" plantain) and D = type of culture (D_1 = SC; D_2 = LAB1 + LAB2; D_3 = LAB1 + LAB3; D_4 = LAB2 + LAB3; D_5 = LAB1 + LAB1 + SC; D_6 = LAB1 + LAB3 + SC; D_7 = LAB2 + LAB3 + SC). Parameters of flour characteristic analyzed or response (Y) were carboxyl number (Y_1), total acid (Y_2), swelling number (Y_3), pH (Y_4), paste clarity (Y_5), gelatinization properties: peak (Y_6); trough 1 (Y_7); breakdown (Y_8); final viscosity (Y_9); setback(Y_{10}); peak time (Y_{11}); pasting temperature (Y_{12}), water content (Y_{13}), color: *L (Y_{14}); *a(Y_{15}); *b (Y_{16}) and c (Y_{17}). The result showed that a combination of 6% culture concentration and 12.62 hours of fermentation time with a desirability value of 0.66 was the optimum condition with carboxyl value 0.15, total acid 0.47, swelling rate 13.49, peak time 5397.02, breakdown 2259.22, final viscosity 4125.22, setback 98.99, and dL 65.59.

Keywords: Modification flour; Optimum condition; Fermentation

INTRODUCTION

Banana is an abundant food ingredient and is commonly consumed by people worldwide. Bananas contain many carbohydrates, with 17.2 - 38% starch content and amylose around 9.1 - 17.2% (Jenie et al., 2012). Generally, there are three types of bananas, namely bananas that can be consumed immediately after ripening, bananas that are consumed after processing (plantain), and bananas that are taken from the leaves and fruit as a source of fibre (seeded bananas) (Nurhayati et al., 2014).

Bananas are included in perishable foodstuffs. Processing banana flour is one way to minimize post-harvest losses while retaining nutrients from fresh bananas (Pragati et al., 2014). The high starch content makes it interesting to use bananas in flour or starch as an intermediate product (Waliszewski et al., 2003). Raw bananas are a source of polyphenol antioxidants that have high activity against cancer prevention, cardiovascular disease, and arthritis (Kumar et al., 2017). Among carbohydrates banana flour contains 73% starch, 17.5% resistant starch, and 14.5% dietary fiber (Pragati et al., 2014). Banana flour's chemical and functional characteristics are limited when used for baking products. Another function is using bananas as food high in resistant starch and prebiotics.

Modification of flour or starch is a way to increase amylose and protein content (Nur Buwono et al., 2018). There are several methods to modify starch, such as physical, chemical, and enzymatic or fermentation (Marta et al., 2019). Physical modifications are generally carried out by heating treatments such as heat moisture treatment (HMT), retrogradation, and annealing (Adiyanti and Subroto, 2020). Chemical modification can be done by oxidation, succinylase, acidification, and esterification. At the same time, the enzymatic modification can be done by spontaneous fermentation or the use of microorganisms

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as a starter. Modification by fermentation is an alternative to changing flour's chemical and rheological characteristics (Haydersah et al., 2012). Spontaneous fermentation can be carried out by soaking the pieces/slices of starchy material in a 3% salt solution for 120 hours (Yuliana et al., 2014), immersion for 24-96 hours in a 1% sugar solution (Yenrina et al., 2015), immersion in water for seven days (Mendes et al., 2019). This method has the disadvantage of uncontrolled chemical changes in the starch structure. Lactic acid bacteria (LAB) and yeast are microorganisms that are commonly used as starters in flour or starch fermentation. The LAB used included *Lactobacillus plantarum* and *Leuconostoc mesenteroides* (Setiarto et al., 2018; Yuliana et al., 2018), *Lactobacillus casei* (Anasiru et al., 2019) and *Lactobacillus plantarum* (Kurniadi et al., 2019). While the yeast used was *Saccharomyces cerevisiae* (Reyes et al., 2016; Yuliana et al., 2018). LAB and yeast were chosen as starters because both microorganisms have passed from a safety point of view (generally recognized as safe/GRAS) (Florou-Paneri et al., 2012; Reyes et al., 2016). Several research report that fermentation by LAB increase the material properties such produce high antioxidant in dough (Sidari et al., 2020), producing of β-Glucan (Allaith et al., 2022), enhance nutritional in food protein and improve its digestibility (Wang et al., 2021), enhance a mineral absorption capacity (Wang et al., 2021).

The fermentation process is influenced by the amount of substrate, the concentration of microorganisms, temperature, length of time, acidity, and the presence of oxygen. Several fermentation factors must be optimized to obtain optimum conditions that produce the desired characteristics of flour/starch. Response Surface Methodology (RSM) is software used to explain the quantitative relationship between input and response variables. RSM has been widely used in optimizing formulations and production processes. Sukasih et al. (2018) used RSM to find the optimal addition of tapioca, citric acid, and banana puree to manufacture instant banana flour. RSM is also used to optimize the temperature and duration of blanching and the concentration of $K_2S_2O_3$ in the pretreatment of banana flour (Fadimu et al., 2018). Another use of RSM is optimizing banana slices' microwave power and drying time (Omolola et al., 2015).

"Tanduk" banana and "Nangka" banana are bananas that can be consumed after going through processing (plantain). In this study, "Tanduk" plantain and "Nangka" plantain flour were modified by fermentation using a mixture of LAB and yeast. This study was to carry out the best condition for making plantain flour by RSM design. The optimized factors were the combination of starter culture, starter concentration, and fermentation time to obtain flour with the best physical, chemical, gelatinization, and thermal properties.

MATERIALS AND METHODS

Materials and methods

The materials used in this study were: "tanduk" (*Musa acuminata* x *Musa balbisiana (AAB) cv. Tanduk*) and "nangka" plantain (*Musa acuminate (AAAcv)* obtained from Pasar Citema of Cibinong, Bogor, West Java. The plantains were sorted by size and ripeness: approximately 30 – 35 cm (l) x 6 – 7 cm (d) x 600 g (weight) for tanduk plantain and 20 – 24 cm (l) 3 – 4 cm (d) x 60 – 75 g (weight) for nangka plantain. Plantain was in green mature and still has hard texture. The microbes employed in this study were *Lactobacillus fermentum* WKS 2 (LAB1), *Lactobacillus plantarum* WKS 4 (LAB2), *Lactobacillus fermentum* WKS 6 (LAB3), and *Saccharomyces cerevisiae* (Sac) laboratory collection. The pro analysis reagent were sodium hydroxide, phenolphthalein, de Mann Rogosa Sharpe Broth (MRSB), Potato Dextrose Broth (PDB) oxalic acid, and others. The equipment used was a Thermo oven (Indelab, Spain), Memmert WNB 14 water bath (Schwabach, Germany), pH meter Eutech Instruments pH 700 (Singapore), UV-Vis spectrophotometer (Shimadzu, Japan), vortex, centrifuge (Hitachi CR21G III, Japan), Rapid Visco Analyzer (RVA-TecMaster, Macquarie Park, Australia), Chrome Meter CR-300, and other glassware. This research was conducted at the Center for Applied Microbiology Research and the Research Center for Appropriate Technology at the National Research and Innovation Agency, Indonesia.

Culture preparation

Aseptically, the LAB stock culture of 5 µl r was grown on 5 ml of MRSB media. Bacterial cultures were incubated at 35 °C for 24 hours. Then 1 ml of the bacterial culture was taken and re-grown into 100 ml of liquid MRS media, then reincubated at 35 °C for 24 hours. This last culture was used as a starter for fermentation. The same work steps were carried out for *S.cerevisiae* using PDB media.

The making of modified plantain flour

The plantain was peeled and then soaked in water to prevent browning. The plantain flesh was sliced thinly (1-2 mm thick) and then put into the fermentation medium with the concentration and type of culture according to the research design. The ratio of plantain slices and fermentation media was 1:3. The fermentation was carried out anaerobically, with a length of time according to the research design.

After the fermentation was complete, the plantain slices were drained and dried immediately. The dried plantain slices were blended and filtered 100 mesh. Then the flour

obtained was analyzed for its physical, chemical, and gelatinization properties.

Analysis

Carboxyl number

Carboxyl number were determined according to method of Smith (1967). The 500 mg flour sample was dissolved in 300 ml of distilled water and boiled for 10 minutes with constant stirring (300 rpm). The solution was added with two drops of 2% (w/v) phenolphthalein indicator. Then titrated with 0.025 M NaOH until the color of the solution is pink. The equation calculates carboxyl content:

$$Carboxyl\ Content\ (\%COOH) = \frac{ml\ NaOH \times 0.025 \times 0.045^{*}}{sample\ weight} \times 100$$

* COOH molecular weight/1000

Total acid

Total acid were evaluated according to Ariantika et al. (2017) with modification. A total of 2.5 g of flour sample was diluted to 25 ml of distillated water, then stirred and filtered using filter paper. The 10 ml sample was added with 2-3 drops of phenolphthalein indicator and titrated with 0.1 M NaOH until the color changed to pink. The equation calculates total acid:

$$Total\ Acid\ (\%) = \frac{(V \times N \times 90 \times FP)}{B \times 1000} \times 100\%$$

Information: V = volume of NaOH used (ml), M = Molarity of NaOH, FP = dilution factor, B = sample weight (g)

Swelling rate

Swelling rate were determined using the method of Schoch (1964). A sample of 1 gram of modified flour was weighed and put in a centrifuge tube that had been weighed previously, added with 50-ml distilled water 50 ml, and gelatinized at 95 °C for 30 minutes with moderate stirring. Then cooled to 30 °C and centrifuged at 2200 rpm for 20 minutes. The gel separated from the aliquots was weighed and then used to calculate the swelling rate. The equation calculates the swelling rate:

$$Swelling\ Rate = \frac{W_2 - W_1}{starch\ weight\ \ (db)}$$

Information: W_1 = weight of centrifuge tube (gram); W_2 = weight of gel and centrifuge tube

pH measurement

After incubation in a certain time, the 50 ml of plantain fermentation medium was checked its pH using the pH meter Eutech Instruments pH 700 (AOAC, 1995).

Gelatinization profile

Gelatinization profile testing was carried out using a Rapid Visco Analyzer (RVA-TecMaster, Macquarie Park, Australia) according to Campuzano et al. (2018). A sample of banana flour was weighed with a weight of 3.5 g (14 g moisture content per 100 g flour) and then mixed with 25 g of distilled water in an aluminum canister. The RVA setting used was the initial temperature of 50 °C maintained for 1 minute, then heated until the temperature reached 95 °C at a speed of 12.2 °C/ minute, then held at 95 °C for 2.5 minutes and cooled to a temperature of 12.2 °C. 50°C at 11.8 °C/min. Paddle rotation speed of 960 rpm in the first 10 seconds then decreases to 160 rpm maintained throughout the analysis process. Parameters recorded include pasting temperature, peak viscosity, final viscosity, breakdown viscosity, and setback viscosity.

Colour

The colour measurement of plantain flour was carried out using a Chroma Meter CR-300 (Konica Minolta Co., Osaka, Japan) with the observed colour parameters L*, a*, and b*. L* indicates the brightness level, which has a scale of 1-100 from black to white; a* value if positive (+) indicates the presence of red, and if negative (-) indicates the presence of green, while the value of b* if positive (+) indicates colour yellow and negative (-) indicates blue.

Research design and data analysis

Optimization of banana flour processing was carried out using RSM with the Design Expert 10 (DX10) application. The design used is Combine D-optimal. The quantitative independent variables (X) selected were A = culture concentration (lower limit 2%; upper limit 6%) and B = fermentation time (lower limit 8 hours; upper limit 24 hours). While the descriptive variables are C = type of plantain (C_1 = "Tanduk" plantain; C_2 = "Nangka" plantain) and D = type of culture (D_1 = SC; D_2 = LAB1 + LAB2; D_3 = LAB1 + LAB3; D_4 = LAB2 + LAB3; D_5 = LAB1 + LAB1 + SC; D_6 = LAB1 + LAB3 + SC; D_7 = LAB2 + LAB3 + SC). The results of randomization can be seen in Table 1.

Parameters of flour characteristic analyzed or response (Y) were carboxyl number (Y_1), total acid (Y_2), swelling number (Y_3), pH (Y_4), paste clarity (Y_5), gelatinization properties: peak (Y_6); trough 1 (Y_7); breakdown (Y_8); final viscosity (Y_9); setback(Y_{10}); peak time (Y_{11}); pasting temperature (Y_{12}), water content (Y_{13}), color: *L (Y_{14}); *a(Y_{15}); *b (Y_{16}) and c (Y_{17}). If the response (Y) is a function of the independent variable (X), as shown by the equation:

Table 1: The research design of optimizing banana flour processing

No.	Culture concentration (%)	Fermentation duration (hours.minutes)	Type of plantain (T=Musa acuminata x *Musa balbisiana (AAB) cv. Tanduk, N : Musa acuminate (AAAcv)*	Culture (%)
1	4.96	23.20	T	*S.cerevisiae* (100)
2	3.59	24.00	N	*S.cerevisiae* (100)
3	5.23	8.00	T	LAB1 (50), LAB2 (50)
4	4.96	8.00	N	LAB1 (50), LAB2 (50)
5	4.99	19.15	T	LAB1 (50), LAB3 (50)
6	3.66	16.36	N	LAB1 (50), LAB3 (50)
7	3.40	8.10	T	LAB2 (50), LAB3 (50)
8	5.27	10.56	N	LAB2 (50), LAB3 (50)
9	5.51	22.29	T	LAB1 (35), LAB2 (35), *S.cerevisiae* (30)
10	3.61	17.35	N	LAB1 (35), LAB2 (35), *S.cerevisiae* (30)
11	5.66	14.08	T	LAB1 (35), LAB3 (35), *S.cerevisiae* (30)
12	4.94	19.44	N	LAB1 (35), LAB3 (35), *S.cerevisiae* (30)
13	3.05	22.41	T	LAB2 (35), LAB3 (35), *S.cerevisiae* (30)
14	2.95	13.06	N	LAB2 (35), LAB3 (35), *S.cerevisiae* (30)
15	4.30	23.06	T	*S.cerevisiae* (100)
16	5.87	15.49	N	*S.cerevisiae* (100)
17	3.81	12.43	T	LAB1 (50), LAB2 (50)
18	2.00	13.32	N	LAB1 (50), LAB2 (50)
19	4.45	17.47	T	LAB1 (50), LAB3 (50)
20	4.39	24.00	N	LAB1 (50), LAB3 (50)
21	4.29	8.00	T	LAB2 (50), LAB3 (50)
22	4.67	19.29	N	LAB2 (50), LAB3 (50)
23	2.10	8.36	T	LAB1 (35), LAB2 (35), *S.cerevisiae* (30)
24	3.93	23.07	N	LAB1 (35), LAB2 (35), *S.cerevisiae* (30)
25	2.93	20.17	T	LAB1 (35), LAB3 (35), *S.cerevisiae* (30)
26	3.16	18.52	N	LAB1 (35), LAB3 (35), *S.cerevisiae* (30)
27	3.86	16.35	T	LAB2 (35), LAB3 (35), *S.cerevisiae* (30)
28	3.68	21.28	N	LAB2 (35), LAB3 (35), *S.cerevisiae* (30)
29	4.85	12.26	T	*S.cerevisiae* (100)
30	4.15	19.32	N	*S.cerevisiae* (100)
31	4.02	24.00	T	LAB1 (50), LAB2 (50)
32	6.00	19.35	N	LAB1 (50), LAB2 (50)
33	3.17	8.34	T	LAB1 (50), LAB3 (50)
34	2.00	14.59	T	*S.cerevisiae* (100)
35	2.31	8.00	N	*S.cerevisiae* (100)
36	2.00	20.00	T	LAB1 (50), LAB2 (50)
37	2.44	24.00	N	LAB1 (50), LAB2 (50)
38	2.06	24.00	T	LAB1 (50), LAB3 (50)
39	5.27	10.56	N	LAB2 (50), LAB3 (50)
40	3.61	17.35	N	LAB1 (35), LAB2 (35), *S.cerevisiae* (30)
41	5.66	14.08	T	LAB1 (35), LAB3 (35), *S.cerevisiae* (30)
42	2.95	13.06	T	LAB2 (35), LAB3 (35), *S.cerevisiae* (30)
43	3.40	8.11	T	LAB2 (50), LAB3 (50)

$$Y = f(X_1, X_2, X_3, \ldots, X_n)$$

Quadratic polynomial model in each response is shown in the equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_1 X_1 + \sum_{i=1}^{3} \beta_{ii} X_{ii}^{\ 2} + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

Whereas β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients, and Xi and Xj are the code levels of the independent variables i and j. The equation to be obtained is in the form of linear, quadratic, or interactive from the independent variables.

Data processing using DX 10. The analysis carried out is the one-way analysis of variance (ANOVA).

RESULT AND DISCUSSION

The optimization results of making modified plantain flour with various types of plantain and mixed culture media of LAB and *S.cerevisiae* covering physical, chemical, and gelatinization characteristics can be seen in Table 2. Table 3 shows the value of the Analysis of Variance (ANOVA) response model for carboxyl numbers, total acid, pH, swelling number, gelatinization profile, moisture content, and colour (L, a, b).

Carboxyl number

The carboxyl number from the optimization of modified plantain flour ranged from 0.117 to 0.172. "Nangka" plantain possessed a higher carboxyl number than the "Tanduk" type. The mixed culture of LAB 1+BAL 3 possessed a higher carboxyl number than other cultures. The culture concentration and fermentation duration also affect the carboxyl number of modified plantain flour. The higher the concentration of culture and the longer the fermentation time, the higher the carboxyl

Table 2: Physical, chemical, and gelatinization properties of plantain flour

No.	CN	TA	SN	pH	P 1	BD	FV	SB	PT	dL*
1	0.132	0.315	12.54	5.95	3239	810	3656	1227	4.9	67.817
2	0.120	0.450	17.66	5.46	5809	2993	3543	727	4.2	64.522
3	0.121	0.225	13.61	5.85	4017	2054	2702	739	4.5	68.920
4	0.124	0.360	17.33	5.49	5858	2398	4321	861	4.7	64.479
5	0.147	0.360	16.13	5.73	4167	1735	3519	1087	4.7	68.783
6	0.173	0.405	17.44	5.42	5368	2079	4099	810	4.9	63.733
7	0.124	0.225	16.68	5.84	3891	1739	2956	804	4.6	68.825
8	0.150	0.405	16.69	5.43	5529	2333	3989	793	4.7	63.941
9	0.158	0.315	15.80	5.87	4530	1783	3993	1246	4.9	67.145
10	0.158	0.405	14.77	5.39	5602	2120	4255	773	4.8	62.954
11	0.132	0.360	17.29	5.84	2814	1138	2002	326	4.5	36.036
12	0.135	0.360	18.45	5.57	4896	2191	3419	714	4.6	64.154
13	0.162	0.315	16.18	5.60	3987	1699	3306	1018	4.8	36.436
14	0.131	0.450	17.54	5.54	4524	1687	3697	860	4.8	62.923
15	0.121	0.270	15.17	5.68	4384	1325	4445	1386	4.9	36.212
16	0.150	0.450	19.23	5.56	5754	2361	4244	851	4.8	63.753
17	0.117	0.270	13.52	5.78	3616	2015	2079	478	4.5	35.895
18	0.131	0.450	17.25	5.40	5625	2216	4362	953	4.8	63.075
19	0.173	0.450	16.02	5.61	3902	1529	3538	1165	4.9	35.729
20	0.139	0.360	19.58	5.47	5005	2587	3128	710	4.4	64.189
21	0.117	0.315	13.74	5.84	4190	806	3483	99	4.6	36.355
22	0.158	0.315	17.63	5.50	4989	2468	3210	689	4.5	57.137
23	0.106	0.180	13.73	5.87	3380	1417	2652	689	4.6	38.856
24	0.143	0.315	18.84	5.37	4461	2152	2917	608	4.5	57.781
25	0.124	0.360	12.42	5.84	4421	1716	3988	1283	4.9	38.563
26	0.158	0.405	17.69	5.45	5727	2249	4405	927	4.7	56.338
27	0.154	0.270	14.48	5.83	4351	1146	4226	1021	4.9	37.713
28	0.143	0.405	18.67	5.46	5092	2362	3508	778	4.6	57.786
29	0.120	0.270	13.18	5.93	3337	1615	2246	524	4.5	37.523
30	0.135	0.360	18.90	5.60	4932	2254	3449	771	4.6	55.661
31	0.150	0.405	16.45	5.73	4367	1538	3766	937	4.9	38.296
32	0.143	0.405	17.76	5.27	5174	2697	3188	711	4.5	56.280
33	0.128	0.315	13.76	5.77	3393	1642	2489	738	4.6	37.990
34	0.120	0.225	14.32	5.96	2940	1484	1946	490	4.4	37.473
35	0.120	0.315	18.93	5.64	4527	2254	2932	659	4.5	57.366
36	0.124	0.360	16.44	5.73	4324	1772	3561	1009	4.9	38.146
37	0.146	0.405	18.45	5.25	5310	2773	3217	680	v	56.753
38	0.120	0.270	13.33	5.83	3703	1798	2708	803	4.7	37.815
39	0.146	0.405	15.86	5.46	5167	2116	3924	873	4.8	56.135
40	0.146	0.405	16.85	5.26	5455	2208	4022	775	4.8	56.065
41	0.124	0.360	16.62	5.85	3366	1719	2139	492	4.5	37.281
42	0.154	0.450	16.88	5.54	6166	2601	4412	847	4.8	56.093
43	0.128	0.225	17.81	5.87	4069	1755	3095	781	4.7	37.972

CN=carboxyl number; TA=total acid; SN=swelling number; P1=peak 1; BD=breakdown; FV=final viscosity; SB=setback

Table 3: ANOVA test results of the mathematical formula model

No.	Response	Mathematic model (*suggested*)	Significance	
			Model	***Lack of fit***
1	Carboxyl number	Linear	significant	Not significant
2	Total acid	Linear	significant	significant
3	Swelling Rate	2F1	significant	not significant
4	pH	Linear	significant	not significant
5	Gelatinization profile			
	a. Peak	Linear	significant	not significant
	b. Breakdown	Linear	significant	not significant
	c. Final viscosity	2F1	significant	not significant
	d. Set back	2F1	significant	significant
6	Colour			
	a. L* (lightness)	Linear	significant	not significant

number (Fig. 1). Similar results were shown in samples of cassava starch fermented with LAB. The presence of a carboxyl group indicates that there is lactic acid content in the final product (Inguillay et al., 2021). Lactic acid is a component consisting of alcohol and a carboxyl group in its chemical structure (Abdel-Rahman et al., 2013). The higher the concentration of culture and the longer the fermentation time, the more the carboxyl number will increase, indicating the higher amount of lactic acid in fermented plantain flour.

The response model of the carboxyl number selected in the optimization of modified plantain flour is linear, meaning that the response is only influenced by each treatment factor but is not influenced by the interaction between factors. This model follows the criteria and has a regression value (R-square) of 0.51, meaning that the treatment factor affects 51% of the carboxyl number of modified plantain flour.

The RSM model or equation for the optimization of the plantain flour modification process on the response to carboxyl numbers is shown in the following equation:

$$\text{Carboxyl Number} = 0.14+8.03A+8.31B +4.85C-0.03D_1-5{,}6D_2+8.4D_3+2.7D_4+2D_5-6D_6 \quad [1]$$

Information:
A = Culture concentrate (%)
B = Fermentation duration (hour)
C = Type of plantain
D = Mixed culture

From equation (1), it is known that the concentration of culture and duration of fermentation gave a positive response, which means that the greater the concentration of culture added and the longer the fermentation time, the greater the carboxyl number. The plantain type factor also responded positively, meaning that selecting the

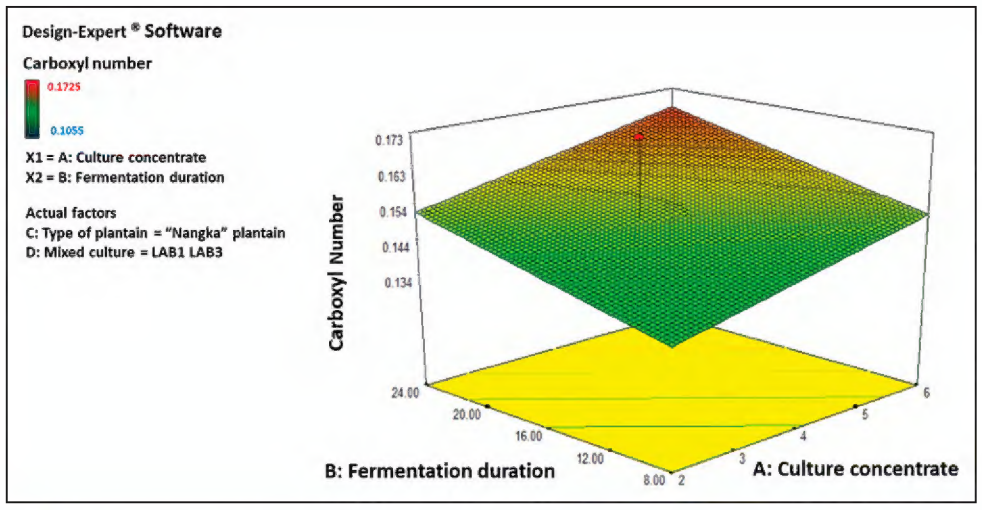

Fig 1. Three-dimensional graph of the carboxyl number response parameter of modified plantain flour.

correct type of plantain will affect the carboxyl number of modified flour. Meanwhile, the culture that gave a positive response was a mixed culture of D_3, D_4, and D_5. The mixed culture of D3 gave the most significant response compared to other cultures. It means that the combination of LAB 1 and BAL 3 produces the most significant lactic acid, where this lactic acid contains of alcohol and carboxyl groups. Ajayi et al. (2019) stated that lactic acid fermentation resulted in changes in several functional groups, which were marked by the presence of hydroxyl, aldehyde, alcohol, and carboxyl groups. Carboxyl number in modification starch by fermentation not only causing by increasing the lactic acid content, it had relationship with molecular fragmentation during oxidation process resulting molecular structure changes in starch. carboxyl content increased at a much higher than carbonyl content, which confirmed that hydroxyl groups on starch molecules were initially oxidized to carbonyl groups and then to carboxyl groups as the primary final product (Putri et al., 2012). In this study, changes in carboxyl numbers were significantly influenced by the concentration of culture and duration of fermentation.

Total acid

The total acid optimization of modified plantain flour ranged from 0.18 to 0.45 (Table 2). The concentration of

culture, duration of fermentation, type of plantain, and type of culture all affected the total acidity of modified plantain flour. The total acid response model selected in the optimization of modified plantain flour is the 2FI (Factorial Interaction) model, meaning that the response is influenced by each treatment factor and is also influenced by the interaction between factors (Table 3). This model follows the criteria and has a regression value (R-square) of 0.91, meaning that the treatment factor affects 91% of the total acidity of modified plantain flour.

The RSM model or equation for the optimization of the plantain flour modification process to the total acid response is shown in the following equation:

$$\text{Total Acid} = 035+0.06A-0.03B+0.05C-0.03D_1+5.35D_2+0.03D_3-0.07D_4+0.02D_5+0.05D_6-8.70AB+5.24AC-0.02AD_1+0.08AD_2+0.03AD_3+0.14AD_4+0.17AD_5-0.10AD_6-0.02BC+0.07BD_1+0.08BD_2+2.86BD_3+0.03BD_4-0.14BD_5-0.07BD_6+0.02CD_1-7.94CD_2-0.03CD_3-0.07CD_4+0.07CD_5-0.02CD_6 \quad [2]$$

Information:
A = Culture concentrate (%)
B = Fermentation duration (hour)
C = Type of plantain
D = Mixed culture

From equation (2), it is known that the concentration factor of the positive response culture means that the greater the concentration of culture added, the greater the total acid. While the long fermentation factor gave a negative response, it means that the longer the fermentation, the total acidity of plantain flour will decrease. The plantain type factor also gave a positive response, meaning that selecting the correct type of plantain will affect the total acidity of the modified flour. The "Nangka"-typed plantain gave a higher total acid response than the "Tanduk"-typed. Meanwhile, the type of culture that gave a positive response was a mixed culture of D_2, D_3, D_5, and D_6. The mixed culture of D_2 gave the most significant response compared to other cultures. The combination of plantain "Nangka" treatment and mixed culture of LAB 1+ LAB2 gave the highest total acid response, 4.50 (Fig. 2).

The trend of total acid different from carboxyl number. It is because the total acid only counting the lactic acid, then the carboxyl is counting the carboxyl group. The carboxyl group not only from lactic acid but also other carboxyl group remain from the hydrolysis of starch (Putri et al., 2012). This study's results align with the research by Jenie et al. (2012) state that the higher the concentration of microbes, the higher the total acid (TA) produced. The TA value increased, indicating that the mixed LAB culture grew

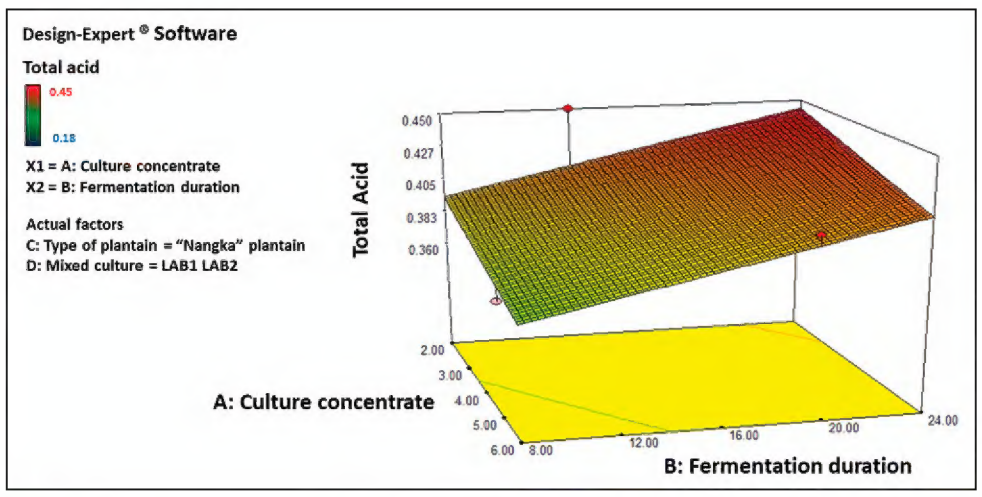

Fig 2. Three-dimensional graph of the total acid response parameter of modified plantain flour.

well on plantain slices and produced lactic acid. The type and duration of fermentation also affect the TA value in this study, which is also in line with the study's results on the fermentation of plantain slices with the peel (Desnilasari et al., 2020). The type of starter affects the amount of acid (TA), it could be increase or decrease, it is due to differences in bacteria fermenting sugar. Based on the ability to ferment sugar, lactic acid bacteria are categorized into two types: homofermentative, which converts sugar into lactic acid, and heterofermentative, which converts sugar into lactic acid, acetic acid, ethanol, and CO_2. The presence of lactic acid produced can reduce environmental pH and increase TA when it is fermented by homofermentative bacteria, but could be decrease when it is fermented by heterofermentative bacteria (Giraffa et al., 2010). The results of the fermentation of "Tanduk" plantains show that the longer the total acid fermentation time increases, the higher lactic acid production (Desnilasari et al., 2020). The fermented "Nangka" plantain gave a higher total acid response because it was suspected that the total acid in the "Nangka" plantain itself was higher than the total acid in the "Tanduk" plantain (0.18%) (Kumalasari et al., 2021).

Swelling rate

Optimizing modified plantain flour resulted in a swelling rate ranging from 12.42% to 19.58% (Table 2). The culture concentrate, duration of fermentation, type of plantain, and type of culture all affected the swelling rate of modified plantain flour. The response model of the swelling rate chosen in the optimization of modified plantain flour is the 2FI (Factorial Interaction) model, meaning that the response is influenced by each treatment factor and is also influenced by the interaction between factors (Table 3). This model follows the criteria and has a regression value (R-square) of 0.95, meaning that the treatment factor has an effect of 95% on the swelling rate of modified plantains.

The RSM model or equation for the optimization of the plantain flour modification process on the response to the swelling rate is shown in the following equation:

$$\text{Swelling rate} = 16.52-1.20A+0.4B+1.75C-0.20D_1 -0.32D_2+0.41D_3+1.25D_4-2.05D_5+1.23D_6-0.48AB +0.51AC+0.95AD_1+0.94AD_2+4.43AD_3- 6.63AD_4-3.58AD_5+2.43AD_6-0.42BC-0.61BD_1 +0.61BD_2-0.37BD_3-0.53BD_4+5.39BD_5-5.50BD_6 +0.77CD_1-30CD_2+0.04CD_3+0.86CD_4- 2.33CD_5+0.83CD_6 \quad [3]$$

Information:
A = Culture concentrate (%)
B = Fermentation duration (hour)
C = Type of plantain
D = Mixed culture

From equation (3), it is known that the concentration factor of the culture response is negative, which means that the greater the concentration of culture added, the smaller the swelling number. The same trend was shown in the fermentation of sago flour (Wahyuni et al., 2021). It could be due to the more significant the concentration of the culture, the greater the amylase enzyme produced to attack the amorphous portion of the starch, which results in the weakening of the starch tissue structure so that the starch expands more easily when heated (Wahyuni et al., 2021). The longer the fermentation, the swelling rate of plantain flour will increase. It is in line with sweet potato flour fermentation (Yuliana et al., 2018), where the increase in swelling ability is due to lactic acid bacteria producing amylase enzymes during fermentation that attack the amorphous portion of starch granules. It further reduces the amylose content and causes the strength of the granule micellar tissue to become less rigid so that it expands more easily when heated in excess water. The plantain type factor also responded positively, meaning that selecting the correct type of plantain will affect the modified flour's swelling rate. The "Nangka"-typed plantain gave a higher swelling response rate than the "Tanduk". It is presumably due to differences in the composition and chain length of amylose-amylopectin, molecular weight, and the molecular confrontation of starch of each type of plantain which gives different degrees of polymerization (Hoover, 2001; Ratnayake et al., 2002).

Meanwhile, the culture that gave a positive response was a mixed culture of D_3, D_4, and D_6. The mixed culture of D_4 gave the most significant response compared to other cultures. The combination of plantain "Nangka" treatment and mixed culture of LAB2+ LAB3 gave the highest swelling response rate, 19.58% (Fig. 3). According to Wahyuni et al. (2021), the type and concentration of lactic acid bacteria varied the swelling value of fermented sago flour, which was influenced by LAB activity.

The stretching of the granule lamellae begins with the absorption of water molecules in the amorphous part of the granule.

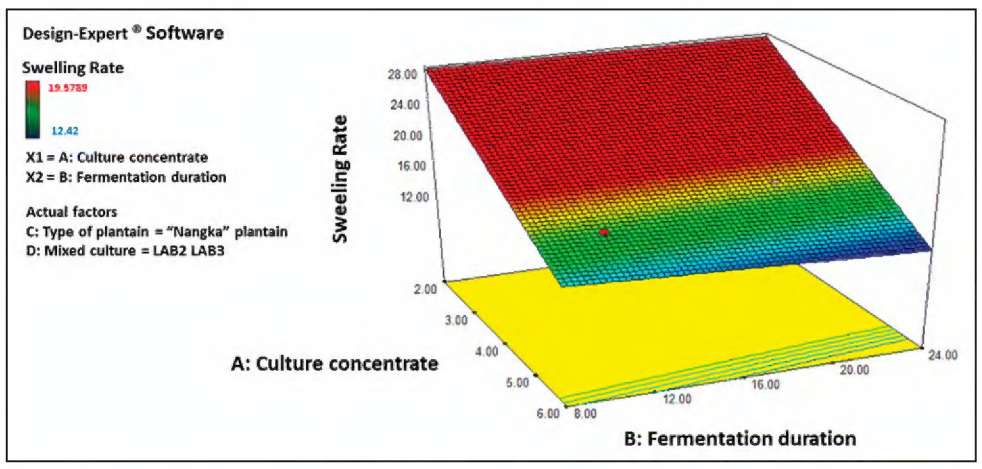

Fig 3. Three-dimensional graphics of modified plantain flour swelling rate response parameter.

pH value

The modified plantain flour optimization pH ranged from 5.29 to 5.96. The "Tanduk"-typed plantain produces a higher pH value than the "Nangka". Saccharomyces type culture produces a higher pH value than other cultures. The culture concentration and fermentation duration also affect the pH value of modified plantain flour. The higher the concentration of culture and the longer the fermentation time, the lower the pH value (Fig. 4).

The response model of the pH value chosen in the optimization of modified plantain flour is linear, meaning that the response is only influenced by each treatment factor but is not influenced by the interaction between factors. This model follows the criteria and has a regression value (R-square) of 0.87, meaning that the treatment factor influences 87% of the pH value of modified plantain flour.

The RSM model or equation for the optimization of the plantain flour modification process to the pH value response is shown in the following equation:

$$\text{pH} = 5.63-3.66A-0.06B-0.17C+0.10D_1-0.07D_2-0.04D_3- 0.015D_4-0.032D_5+0.05D_6 \quad [4]$$

Information:
A = Culture concentrate (%)
B = Fermentation duration (hour)
C = Type of plantain
D = Mixed culture

Equation (4) shows that the concentration factor of culture and duration of fermentation is negative, which means that the greater the concentration of culture added and the longer the fermentation, the lower the pH value. According to Yuliana et al. (2018), fermentation resulted in a decrease in pH and an increase in the TA value. The longer the fermentation, the smaller the pH in line with the results of the decrease in pH in sweet potato flour fermentation caused by the increasing amount of lactic acid produced during fermentation (Yuliana et al., 2018). The

"Tanduk" plantain gave a higher pH value response than the "Nangka". The acid content can influence it in each plantain before fermentation. Raw "Tanduk" plantains have a pH of 6.13 (Kumalasari et al., 2021), which is thought to be higher than the pH of "Nangka" plantains.

Meanwhile, the culture that gave a positive response was a mixed culture of D_1 and D_6. The mixed culture of D_1 gave the most significant response compared to other cultures. According to Handayani et al. (2022), different types of cultures produce different pH related to the presence of organic acids produced during fermentation.

Gelatinization profile

The most important functional properties of flour or starch are thermal and paste. Based on the pasta graph, several parameters can be investigated, such as the possibility of disintegration, when gelatinization occurs and the possibility of retrogradation. Viscosity is related to molecular weight, granule composition, pH, and electrolyte properties of the solution. The presence of other components such as protein and fat in flour will affect the initial temperature and gelatinization so that the flour paste's nature will differ from the starch pastes. Differences in the origin of starch will also distinguish the nature of the paste. Each starch granule does not continually expand at the same temperature, the origin of starch and the presence of protein, fat, and sugar content will cause differences in the pattern of expansion.

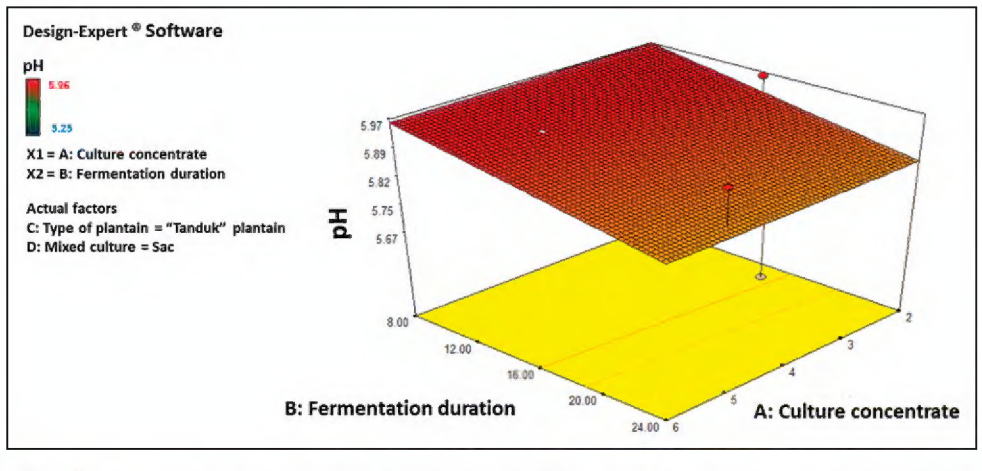

Fig 4. Three-dimensional graph of the response parameter of the modified plantain flour pH value.

The peak response model selected for modified plantain flour optimization is linear. The treatment factor affects 74% of the peak of modified plantain flour. The optimization equation for the modified plantain flour process on the peak response is shown in the following equation:

$$\text{Peak} = 4547.3+30.67A+148.9B+701.9C213.03D_1 +237.7D_2-97.83D_3+180D_4-32.28D_5-195.9D_6 \quad [5]$$

Information:
A = Culture concentrate (%)
B = Fermentation duration (hour)
C = Type of plantain
D = Mixed culture

Equation (5) shows that the concentration of culture and fermentation duration positively affect the peak of modified plantain flour. The greater the concentration of culture added and the longer the fermentation, the higher the peak viscosity. It is in line with the increase in viscosity in the fermentation of sorghum flour (Kurniadi et al., 2019). According to Armada and Putri (2016), this phenomenon occurs due to starch degradation by microbes. There is a separate and soluble amylose fraction in the fermentation medium. Less amylose or higher amylopectin content resulted in starch tending to absorb more water to be more viscous. The "Nangka"-typed plantain gave a higher peak viscosity response than the "Tanduk". It could be due to differences in composition, chain length, and molecular weight of amylose and amylopectin in the two types of plantains.

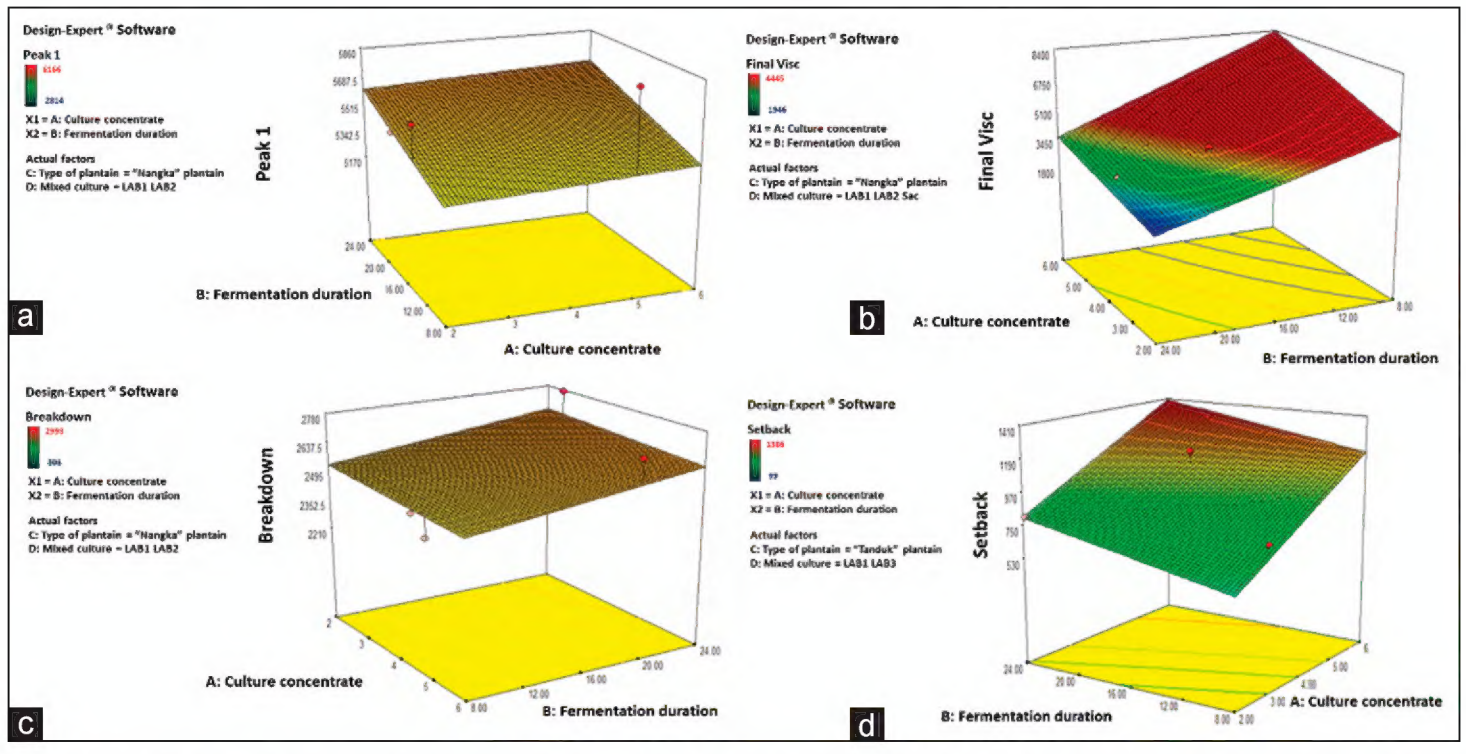

Fig 5. Three dimensional graph of modified plantain flour gelatinization profile response parameters (a = peak, b = breakdown, c = final viscosity, d = setback).

Meanwhile, the culture that gave a positive response was a mixed culture of D_2 and D_4. Mixed culture D_2 (LAB1 + LAB2) gave the most significant response compared to other cultures. It indicates that the mixed culture has high microbial activity during fermentation, resulting in fibre degradation and starch hydrolysis, increasing peak viscosity. These results are similar to those reported by Kresnowati et al. (2019) on cassava flour fermentation.

The breakdown response model selected for optimization of modified plantain flour is linear. The treatment factor affects 70% of the breakdown of modified plantain flour. The optimization equation for the modified plantain flour process on the breakdown response is shown in the following equation:

$$\text{Breakdown} = 1926.81-21.77A+65B+393.8C53.23D_1 +252.11D_2+77.9D_3-12.16D_4-87.14D_5- 51.87D_6 \quad [6]$$

Information:
A = Culture concentrate (%)
B = Fermentation duration (hour)
C = Type of plantain
D = Mixed culture

Equation (6) shows that the length of fermentation positively affects the breakdown of modified plantain flour. The longer the fermentation, the greater the breakdown value, which indicates the plantain flour is unstable during heating. The results of this study follow the results of research by Desnilasari et al. (2020). On the other hand, the greater the concentration of added culture, the lower the breakdown value. "Nangka" plantain provides a higher breakdown response value than the "Tanduk"-typed. It is influenced by the gelatinization profile of "Tanduk" plantains and "Nangka" without modification, where "Tanduk" plantain flour has a higher BD value (0.34 Pa.s) than the BD value of "Nangka" plantain flour (0.12 Pa.S) (Cheok et al., 2018).

Meanwhile, the culture that gave a positive response was a mixed culture of D_2 and D_3. Mixed culture D_2 (LAB1 + LAB2) gave the most significant response compared to other cultures. It is due to the difference in activity resulting from the mixed culture.

The final viscosity response model selected for optimization of modified plantain flour is 2FI (factorial interaction). The treatment factor has an effect of 93% on the final viscosity of modified plantain flour. The optimization equation for the modified plantain flour process on the final viscosity response is shown in the following equation:

$$\text{Final viscosity} = 3781.73+705.34A-180.97B+320.6C-644.3D_1-458.10D_2-15.20D_3+59.3D_4+441.6D_5-258.95D_6-362.99AB-48.69AC-21.7AD_1-812.22AD_2+214.8AD_3-23.35AD_4+535.7AD_5-1558.08AD_6-746.7BC+879BD_1+184.51BD_2-426.6BD_3+592.9BD_4-1113.8BD_5+457.7BD_6+152CD_1+96.6CD_2+276.6CD_3-964.2CD_4+218.3CD_5+296.8CD_6 \quad [7]$$

Information:
A = Culture concentrate (%)
B = Fermentation duration (hour)
C = Type of plantain
D = Mixed culture

Equation (7) shows that the culture concentration factor positively affects the final viscosity of modified plantain flour. The greater the concentration of culture added, the lower the final viscosity value. It is due to the acid hydrolysis produced by lactic acid bacteria to the amorphous starch region resulting in lower molecular weight (Dutta et al., 2011). The "Nangka"-typed plantain gives a higher final viscosity value response than the "Tanduk"-typed. Differences influence the composition, chain length, and molecular weight of amylose and amylopectin in the two types of plantains. According to Cheok et al. (2018), "Tanduk" and "Nangka" flour have different gelatinization profiles, giving different changes after the fermentation treatment.

Meanwhile, the culture that gives a positive response is a mixed culture of D_4 and D_5. Mixed culture D_5 (LAB1 1+ LAB2+Sacharomyces) gave the most significant response compared to other cultures. It indicates that the mixed culture has high microbial activity.

The setback response model selected for optimization of modified plantain flour is 2FI (factorial interaction). The treatment factor has an effect of 92% on the setback of modified plantain flour. The optimization equation for the modified plantain flour process on the final viscosity response is shown in the following equation:

$$\text{Setback} = 849.44-141.31A+53.26B+71.19C-99.15D_1-56.45D_2+94.33D_3-28.36D_4+71.87D_5-134.17D_6-9.17AB-41.29AC+261.27AD_1+113.65AD_2+405AD_3-1.476.25AD_4+265.74AD_5-98.89AD_6-206.74BC+228.79BD_1-26.98BD_2-132.40BD_3-443.87BD_4-105.82BD_5+521.90BD_6-75.19CD_1-62.88CD_2-103.18CD_3+609.51CD_4-151.29CD_5-111.04CD_6 \quad [8]$$

Information:
A = Culture concentrate (%)
B = Fermentation duration (hour)
C = Type of plantain
D = Mixed culture

Equation (8) shows that the concentration of culture has a negative effect on the setback of modified plantain flour. The setback viscosity is related to the amylose composition of the flour. It indicates the tendency of the flour to retrograde (Niba et al., 2002). The higher the SB value, the higher the tendency for retrogradation to occur (Babu et al., 2014). The greater the concentration of culture added, the smaller the setback value. It means that the tendency to experience retrogradation is low. It is in line with the results of the research reported by Buwono et al. (2018), which can be caused by the acidic conditions formed, resulting in starch molecules not having sufficient time to bind to each other during the specified period.

On the other hand, the longer the fermentation, the higher the setback value. It contrasts the fermented plantain with peel reported by (Desnilasari et al., 2020). The "Tanduk"-typed plantain gave a higher setback value response than the "Nangka". This response can be influenced by the gelatinization profile of plantain "Tanduk" flour, which has a higher setback value (0.45 Pa.s) than the setback value of "Nangka" plantain flour (0.34 Pa.S) (Cheok et al., 2018). Meanwhile, the culture that gave a positive response was a mixed culture of D_3 and D_5. Mixed culture D_3 (LAB1+ LAB3) gave the most significant response compared to other cultures. It indicates that the activity of this mixed culture produces plantain flour with a high tendency for retrogradation.

Color (Brightness/L*)

The value of L* or the brightness of modified plantain flour as a result of optimization ranged from 35.73 to 68.92. The higher the L value, the brighter the colour of the plantain flour produced. "Nangka"-typed plantain produces a higher L* value than the "Tanduk"-typed. It can be influenced by the content of polyphenols enzyme, free sugars, and proteins in plantains, which trigger an enzymatic browning reaction during fermentation. Cultures of LAB1+LAB2+Sacharomyces possessed d a higher L* value than other cultures. It can be caused by differences in the activity of the given culture in producing organic acids. According to Anyasi et al. (2017), pretreatment using organic acids in the manufacture of raw plantain flour can reduce browning so that the resulting flour has a lighter colour which increases L* indigo. Culture concentration and fermentation time also affect the L* value of modified plantain flour. The higher the culture concentration and the shorter the fermentation time, the higher the L* value (Fig. 6). It is different from the results of fermentation in sorghum flour, where the concentration and duration of fermentation do not affect the intensity of the colour of the flour. According to Seveline et al. (2020), the fermentation process triggers the pigment degradation process in food products. During the immersion process, the colour components are damaged and increase in whiteness and brightness (Amanu and Susanto, 2014).

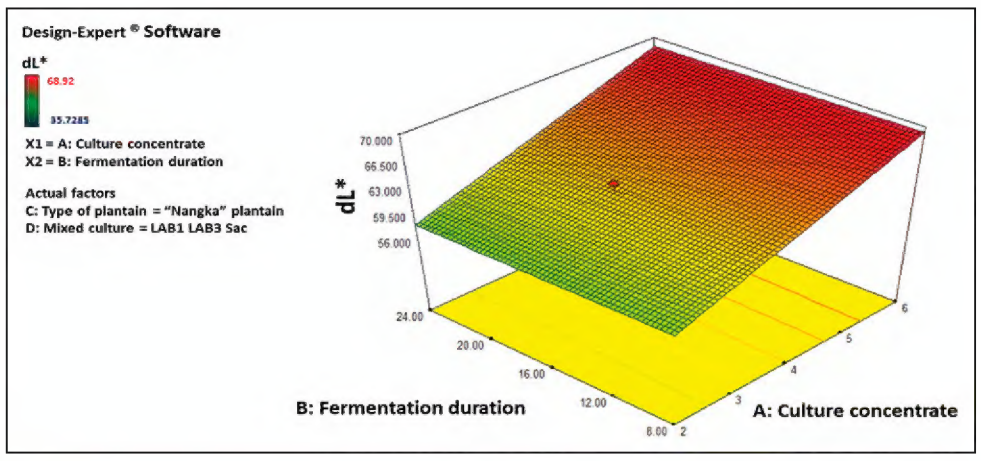

Fig 6. Three-dimensional graph of the response parameter L* value of modified plantain flour.

The response model of the L* value chosen in the optimization of modified plantain flour is linear, meaning that the response is only influenced by each treatment factor but is not influenced by the interaction between factors. This model follows the criteria and has a regression value (R-square) of 0.49, meaning that the treatment factor affects 49% of the L* value of modified plantain flour.

The RSM model or equation for the optimization of the plantain flour modification process to the L* value response is shown in the following equation:

$$L^* \text{ Value} = 52.26+5.61A-0.18B+7.69C+0.31D_1+1.02D_2 +2.33D_3-0.05D_4+3.51D_5-5.52D_6 \quad [9]$$

Information:
A = Culture concentrate (%)
B = Fermentation duration (hour)
C = Type of plantain
D = Mixed culture

Determination of optimum conditions

Design-Expert chose a combination of 6% culture concentration and 12.62 hours of plantain slices fermentation time with a desirability value of 0.66. The predictive desirability value of this program is not close to 1. It can be seen from several verification values that are not correct from the predicted value. Several studies related to the fermentation process using expert designs have low desirability values because there are too many variables and responses used, so further research is needed by focusing on the main variables and responses to obtain optimal results. The closer the value is to 1, the model shows the accuracy of the optimization. The type of plantain selected was the "Nangka" variety and the bacterial culture selected was a mixed culture of LAB2+BAL3. The results of verifying the optimum combination of treatments can be seen in Table 4.

Table 4: Response to verifying the treatment combination model in optimizing modified plantain flour

Response	Prediction	Verification	95% CI low	95% CI high	95% PI low	95% PI high
Carboxyl value	0.15	0.46	0.14	0.16	0.12	0.18
Total acid	0.478	0.61	0.36	0.6	0.33	0.63
Swelling rate	13.49	6.35	10.89	16.09	10.29	16.69
Peak 1	5397.02	7686.25	4894.4	5899.65	4250.12	6543.93
Breakdown	2259.25	4043	1951.32	2567.18	1556.61	2961.88
Final Viscosity	4125.22	4523.75	3064.16	5186.27	2818.83	5431.61
Setback	98.9943	880.5	-291.61	489.6	-381.92	579.91
dL*	65.59	56.23225	55.56	75.62	42.7	88.48

CONCLUSION

This research has predicted mathematically the optimum process for fermentation of plantain flour with a good fit. Design-Expert chose a combination of 6% culture concentration and 12.62 hours of fermentation time with a desirability value of 0.66. The type of plantain selected was the "Nangka" variety, and the bacterial culture was a mixed culture of LAB2+LAB3. This give the prediction response carboxyl value 0.15, total acid 0.47, swelling rate 13.49, peak time 5397.02, breakdown 2259.22, final viscosity 4125.22, setback 98.99, and dL 65.59.

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